CC 14(Recombinant DNA technology)

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Bacterial transformation is a key step in molecular cloning, the goal of which is to produce multiple copies of a recombinant DNA molecule. In **transformation**, the DNA (usually in the form of a plasmid) is introduced into a competent strain of bacteria, so that the bacteria may then replicate the sequence of interest in amounts suitable for further analysis and/or manipulation.

Competent cells are bacterial cells that can accept extra-chromosomal DNA or plasmids (naked DNA) from the environment.

The generation of competent cells may occur by two methods:

1. natural competence
2. Artificial competence.

Natural competence is the genetic ability of a bacterium to receive environmental DNA under natural or in vitro conditions. Bacteria can also be made competent artificially by chemical treatment and heat shock to make them transiently permeable to DNA. Natural competence dates back to 1928 when Frederick Griffith discovered that prepared heat-killed cells of a pathogenic bacterium could transform the nonpathogenic cells into pathogenic type. Natural competence has been reported in many bacterial strains, i.e. *Bacillus subtilis*, *Streptococcus pneumonia, Neisseria gonorrhoeae* and *Haemophilus influenza.*

Artificial competence is not coded by the genes of the bacterial cells. It is a laboratory procedure in which cells are passively made permeable to DNA using unnatural conditions. The procedure of artificial competence is relatively simple and easy and can be used to engineer a bacterium genetically. However, transformation efficiency is very low as only a portion of the cells become competent to successfully take up DNA.

Artificial Competence and Transformation

Artificial competence is not encoded in the cell's genes. Instead, it is a laboratory procedure by which cells are made permeable to DNA, with conditions that do not normally occur in nature. This procedure is comparatively easy and simple, and can be used in the genetic engineering of bacteria but in general transformation efficiency is low.
Methods for preparing the competent cells derive from the work of Mandel and Higa who developed a simple treatment based on soaking the cells in cold $CaCl_2$.

There are two main methods for the preparation of competent cells.They are Calcium chloride method and Electroporation.
Rapidly growing cells are made competent more easily than cells in other Growth stages. So it is necessary to bring cells into log phase before the procedure is begun. The cells in rapid growth (log phase) are living, healthy, and actively metabolizing. Competent cells are readily available in commercial markets.

Reagents Required and Their Role Luria-Bertani Broth

Luria-Bertani (LB) broth is a rich medium that permits fast growth and good growth yields for many species including E. coli. It is the most commonly used medium in microbiology and molecular biology studies for E. coli cell cultures. Easy preparation, fast growth of most E. coli strains, ready availability and simple compositions contribute to the popularity of LB broth. LB can support E. coli growth (OD_{600} = 2–3) under normal shaking incubation conditions.

Calcium Chloride

Calcium chloride transformation technique is the most efficient technique among the competent cell preparation protocols. It increases the bacterial cell's ability to incorporate plasmid DNA, facilitating genetic transformation. Addition of calcium chloride to the cell suspension allows the binding of plasmid DNA to LPS. Thus, both the negatively charged DNA backbone and LPS come together and when heat shock is provided, plasmid DNA passes into the bacterial cell. Prepare 2000 ml of 50 mM Calcium
chloride stock solution by adding 14.701 g of $CaCl_2.2H_2O$ in 2 l of milli-Q water, autoclave, and store at 4 °C.

Materials

1. LB broth: Yeast extract 0.5%, NaCl l%, tryptone 1%.
   2 LB agar: As above, plus 2% agar prior to autoclaving.
3. 0.1M $CaCl_2$.

Antibiotics are added to the above media after autoclaving. Tetracycline to a final concentration of 15 pg/mL and ampicillin to 50 kg/mL Solutions of these antibiotics are prepared with ampicillin at 50 mg/mL m slightly alkaline distilled water and tetracycline at 15 mg/mL in ethanol.

Method

1. Prepare a small, overnight culture of the bacteria in LB broth. Grow at 37°C without shaking.
2. About 2 h before you are ready to begin the main procedure, use 1.0 mL of the overnight culture to inoculate 100 mL of fresh LB broth. This culture is grown with rapid shaking at 37°C until it reaches roughly 5 x 107 cells/ml. Thus corresponds to an OD650 for our cultures, but you should calibrate this for each of your own strains
3. Take a 5 mL aliquot of each transformation reaction and transfer to sterile plastic centrifuge tubes. Cool on ice for 10 mm.
4. Pellet the cells by spinning for 5 mm at 5000g. It is necessary for the centrifugation to be performed at 4°C. We have found a refrigerated bench centrifuge ideal for this.
5. Pour off the supernatant and resuspend cells in 25 mL of cold 0.1M CaCl2. Leave on ice for at least 20 min.
6. Centrifuge as in Step 3. You should observe a more diffuse pellet than previously. This is an indication of competent cells.
7. Resuspend the cells in 0.2 mL of cold 0.1M CaCl2.
8. Transfer the suspensions to sterile, thin-walled glass bottles or tubes. The use of glass makes the subsequent heat shocks more effective.
9. To each tube add up to 0.1 mg of DNA, made up in a standard DNA storage buffer such as TE to a volume of 100 mL. Leave on ice for 30 min.
10. Transfer to a 42°C water bath for 2 min and return briefly to ice.
11. Transfer the contents of each tube to 2 mL of LB broth in a small flask. Incubate with shaking at 37°C for 60-90 min.
12. Plate 0.1 mL aliquots of undiluted, 10-1 and 10-2 dilutions onto LB plates to which the antibiotics to be used for selection have been added.
13. Incubate overnight at 37°C.

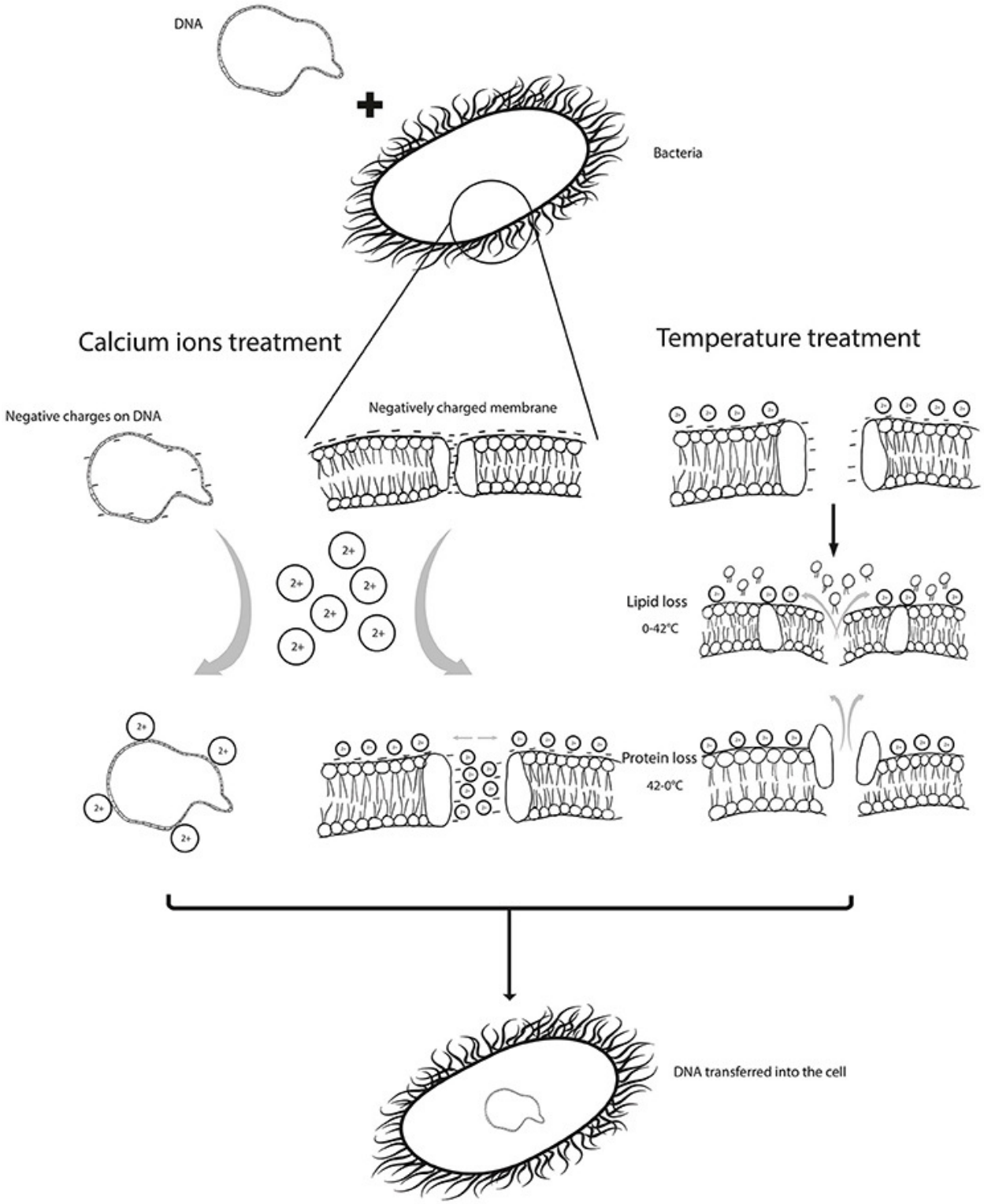
NOTE:

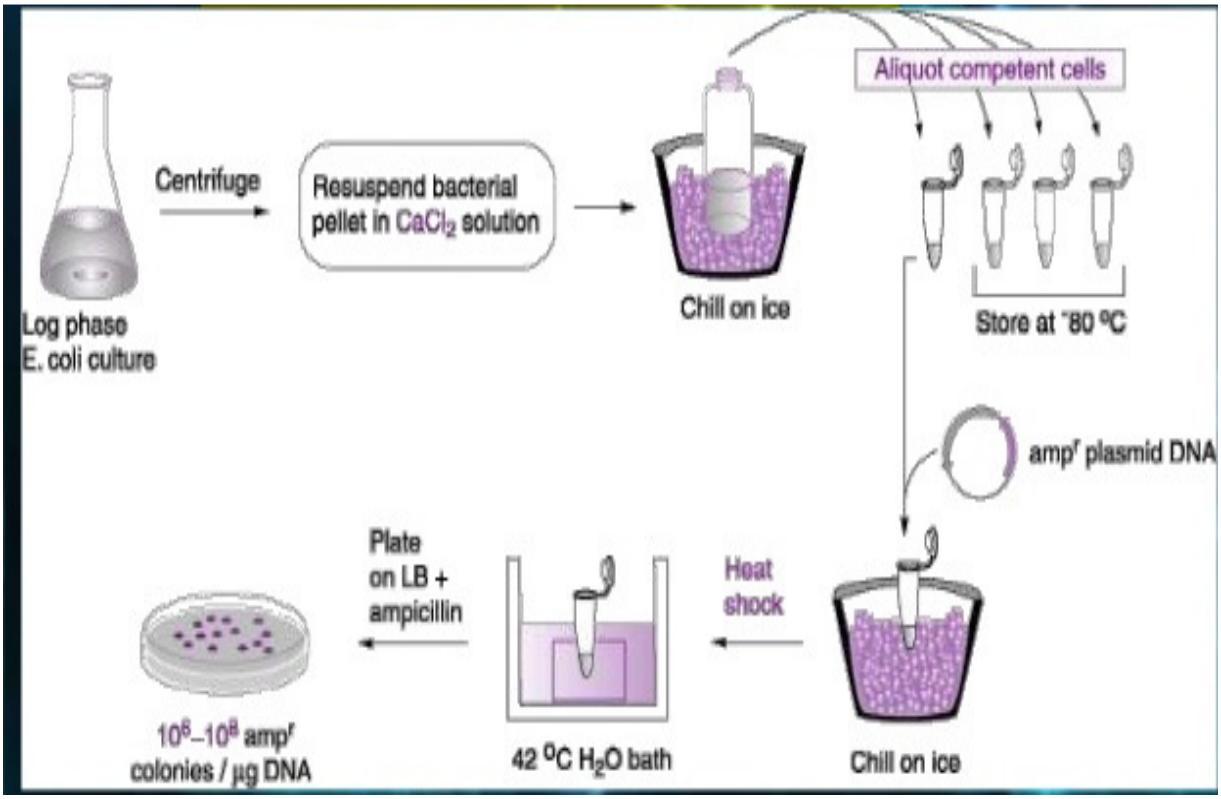
1. This method generally gives 104-106 transformants/mg of closed circle plasmid DNA. Do note that the relationship between amounts of DNA added and yield is not totally linear. Greater than 0.1 mg of plasmid DNA per tube will decrease transformation efficiency.

2. It is essential that the cells used are in a rapid growth phase when harvested. Do not let them approach stationary phase.

3. Cells can be stored at 4°C once competent. Holding cells in CaCl2 at 4°C will, in fact, increase transformation efficiency although this declines with more than 24 h storage. Long periods of storage can be achieved by freezing the competent cells.
4. The revival step is necessary both to allow the plasmid establishment and to allow expression of the resistance genes.
5. One problem encountered on plating on ampicillin is that resistant colonies will often be surrounded by a region of secondary growth. This is caused by the p-lactamase activity of the resistant cells hydrolyzing the surrounding antibiotic and thus allowing surviving sensitive cells to begin to grow. This problem can be avoided by using freshly made ampicillin plates and removing plates from the incubator promptly after the period of overnight growth.

ACTION OF CALCIUM CHLORIDE :





GENE DELIVERY :

a. Microinjection :

Microinjection is most successfully and widely used technique for gene transfer in fish. One method of microinjection technique involves the use of fine injection needle for introducing DNA into cut site in the cell. While doing so it destroys those cells that are in direct contact with the injected DNA.

Advantages of Microinjection Technique:

This technique has the following merits:

(1) Optimum quantity of DNA can be delivered per cell, increasing chances for integrative transformation.

(2) The delivery of DNA is precise, even into nuclei of target cell again improving chances for integrative transformation.

(3) The small structure can be injected.

(4) It is a direct physical approach, hence it is a host range independent.

Disadvantages of Microinjection Technique:

(1) A single cell can be injected at a time, hence it is time consuming process.

(2) It requires sophisticated instruments and specialized skills.

(3) low transformation rate.

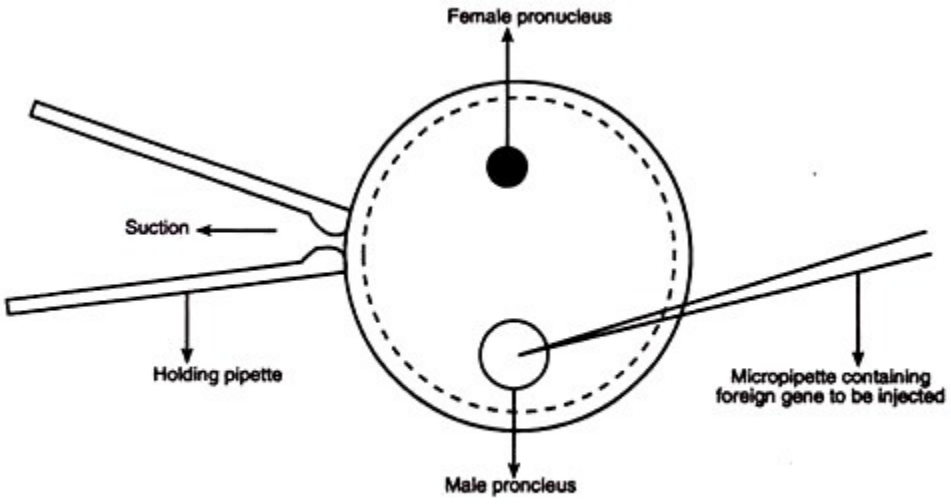


Fig. 43.2 : Micro injection appartus to introduce DNA in intact Cell

Gene Transfer by Electroporation:

It is a simple, fast, efficient and convenient method for transferring gene. This method involves an electrical pulse to deliver DNA into cells . The cells are exposed to a short electrical shock, which make the cell membrane temporarily permeable to DNA.

The desired DNA fragment is placed in direct contact of protoplast membrane, which enters into the cell upon electric shock. Hole may be created as a result and stabilized by a favourable dipole interaction with electric field.

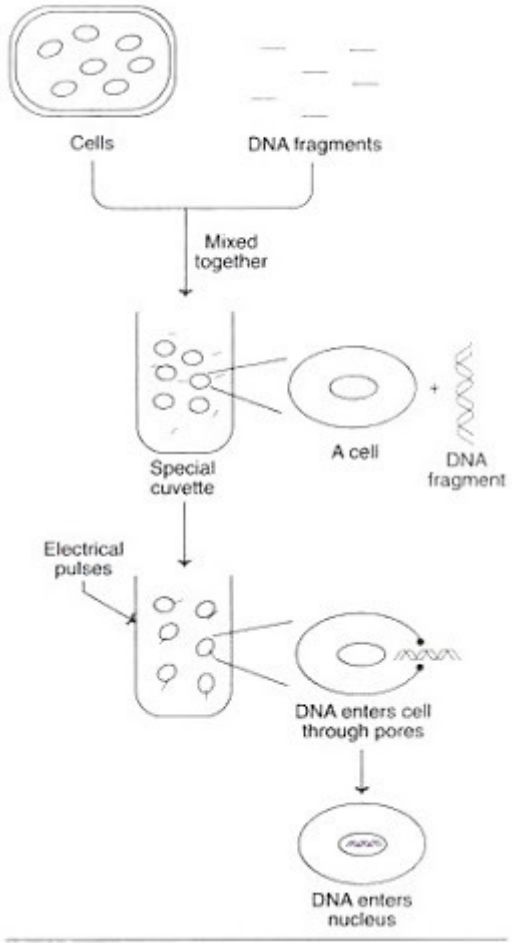
Electroporation involves a chain of electrical pulses for permeation of cell membrane, thereby allowing the entry of DNA into the cell. The rate of DNA integration in electroporated cell is more than 25% is the surviving rate, which is slightly higher in comparison of microinjected ones.

Advantages of Electroporation Technique:

(1) It allows simultaneous entry of DNA constructs.

(2) It is more suitable method for those species, which has very small eggs for microinjection.

(3) This method does not require specialized skill.



Electroporation

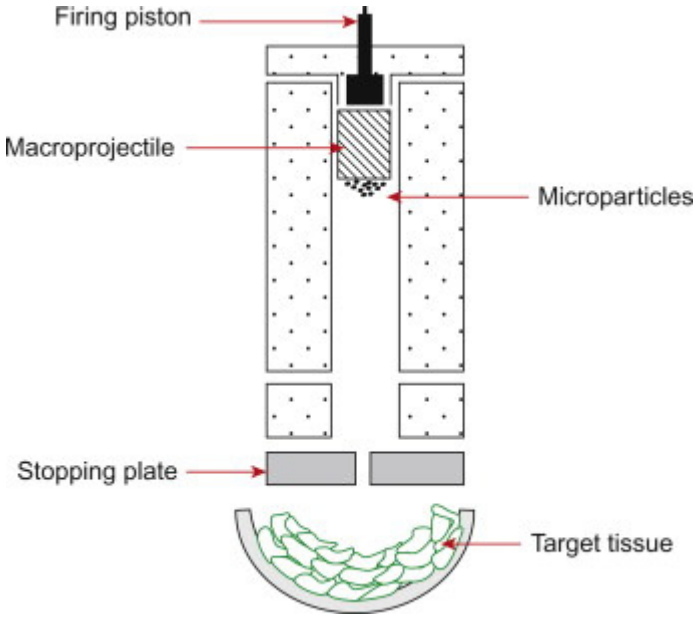
BIOLISTIC METHOD OF GENE DELIVERY(GENE GUN):

Biolistics is a method for the delivery of nucleic acid to cells by high-speed particle bombardment. The technique uses nucleic acid-coated particles propelled by a pressurized gun (gene gun) to transfect cells or organelles. It can also be used to deliver vaccines.

ADVANTAGE:

1. It facilitates direct transfer of the gene in various tissues, overcoming barriers such as the cell wall.

2. this gene transfer is not affected by the kinds of cell, receptors, and molecules at the cell surface



LIPOSOME MEDIATED GENE DELIVERY:

Liposome and lipoplex method-Another approach of DNA transfection in animal cell is to pack the DNA in a lipid vesicle or liposome. In this approach, DNA containing vesicle will be fused with the cell membrane and deliver the DNA to the target cell.

Preparation of liposome and encapsulating DNA was a crucial step to achieve good transfection efficiency. Liposome prepared with the cationic or neutral lipid facilitates DNA binding to form complex (lipoplex) and allow uptake of these complexes by endocytosis.

The lipoplex method was applicable to a wide variety of cells, and found to transfect large size DNA as well. Another advantage of liposome/lipoplexes is that with the addition of ligand in the lipid bilayer, it can be used to target specific organ in the animal or a site within an organ.

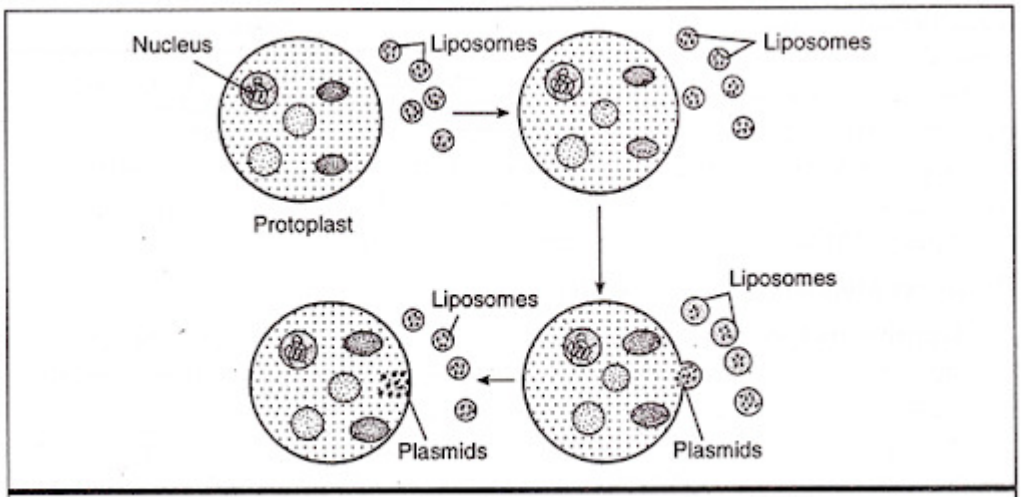
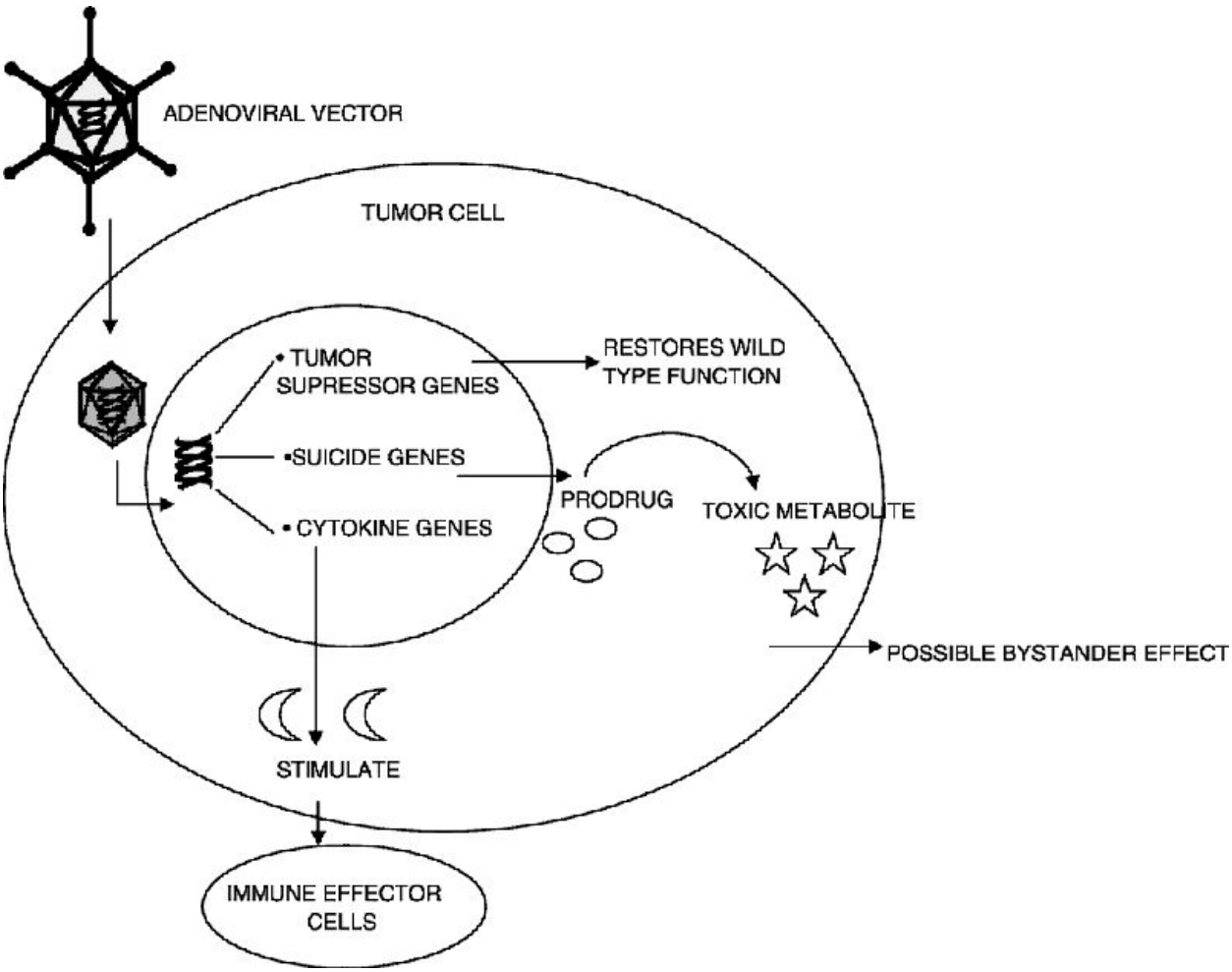


Fig. 24.7B: Fusion of plasmid-filled liposomes with protoplast

VIRUS MEDIATED GENE DELIVERY- Viral particle has a natural tendency to attack and deliver the DNA into the eukaryotic cells. As discussed previously, cloning gene of interest in to the viral vectors is a innovative way to deliver the DNA into the host cell. If the recombination sequences are available, the delivered DNA is integrated into the host and replicate. Virus has essential components for expression of proteins required for DNA replication, RNA polymerase and other ligand for attachment onto the host cell. In addition, it has additional structural components to regulate infection cycle. The virus vector contains cassettes to perform all these functions then it is fully sufficient to propagate independently. Few virus strains may cause disease if their propagation will be uncontrolled.

A mechanism has been devised to keep a check on the uncontrolled propagation of virus in cell. Few crucial structural blocks are placed on another helper plasmid, in this case virus propagate only if helper plasmid has been supplied along with the viral vector. This particular arrangement is made with the virus strains which can cause disease after integrating into the genome such as lentivirus.



VIRUS MEDIATED GENE TRANSFER

AGROBACTERIUM MEDIATED GENE DELIVERY:

Agrobacterium tumefaciens is a soil phytopathogen that naturally infects plant wound sites and causes crown gall disease via delivery of **transferred** (T)-**DNA** from bacterial cells into host plant cells through a bacterial type IV secretion system (T4SS)

This DNA segment (transfer DNA or T-DNA) is present on large plasmid called Tumor- inducing (Ti) plasmids in the bacterium. The T-DNA (about 20 kb long) is integrated into the plant chromosome by recombination. A series of virulence (vir)

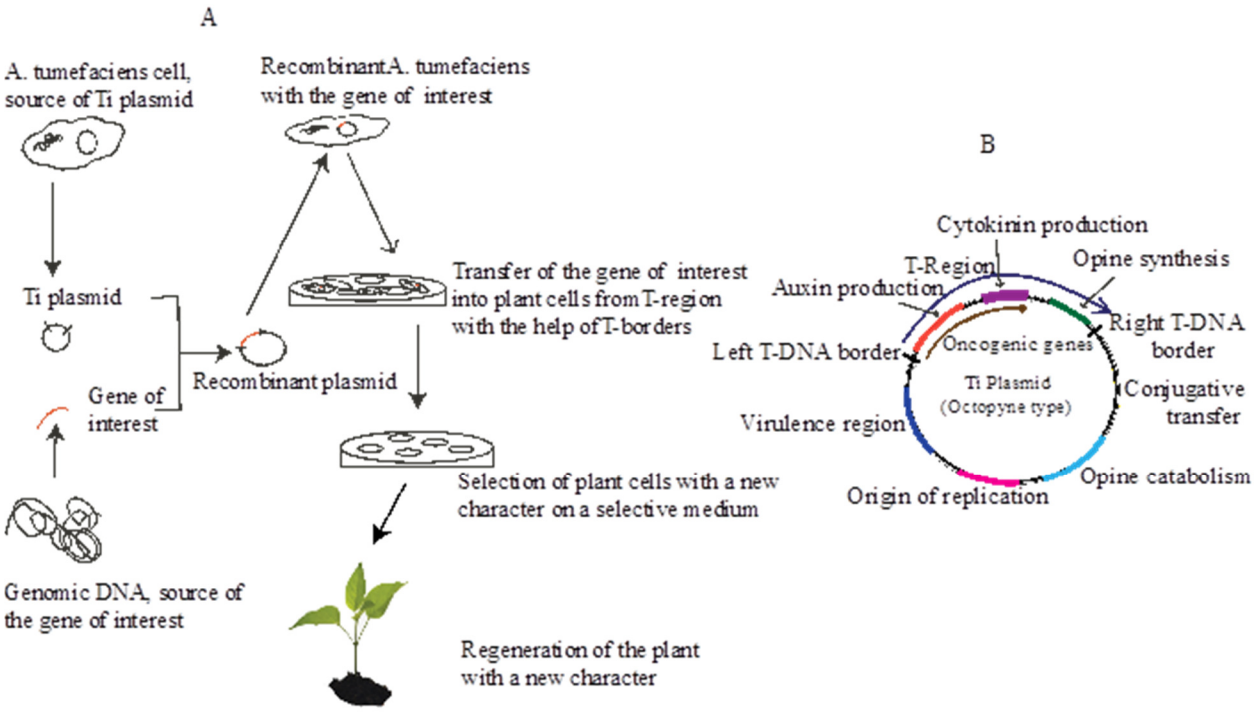
genes are involved in directing the infection process. So when a plant root or a stem is wounded it gives off certain response.

In response to those signals, the vir genes of A. tumefactions become activated, and direct a series of events required for the transfer of the T-DNA from the Ti plasmid to the plant's chromosome. The function of different vir genes include a copy of T-DNA, followed by attachment of a product to the copied T-DNA strand to act as a leader, subsequently add proteins along with the length of the T-DNA, possibly as a protective mechanism.

These eventually open a channel in the bacterial cell membrane, through which the T-DNA passes. The T-DNA then enters the plant through the wound. However, it still is not clear how the bacterial DNA moves from the cytoplasm to the nucleus of the plant cell, or how the T- DNA becomes integrated into the plant chromosomes.

To use these bacteria as a vector its T-DNA region is removed excepting the border regions and the vir genes. The transgene is then inserted between the T-DNA regions, where it is transferred to plant cell and becomes integrated into the plant's chromosome The T-DNA is cloned in Ti-plasmids, which are cut to size and replicated in E.coli to facilitate further manipulation. These vectors are mobilized into Agrobacterium host strains and used to infect the plant tissues.

These infected plant tissues are subsequently grown on media containing specific chemicals, growth regulators to facilitate the regeneration of transformed cells. The selection of trans-formants is done in presence of an antibiotic present in the culture medium. The transformed plants are eventually analysed for stable integration and functional analysis of the inserted gene


A
A. tumefaciens cell, source of Ti plasmid
RecombinantA. tumefaciens with the gene of interest
Ti plasmid
Transfer of the gene of interest into plant cells from T-region with the help of T-borders
Recombinant plasmid
Gene of interest
Selection of plant cells with a new character on a selective medium
Genomic DNA, source of the gene of interest
Regeneration of the plant with a new character
B
Cytokinin production
T-Region
Opine synthesis
Auxin production
Right T-DNA border
Left T-DNA border
Oncogenic genes
Ti Plasmid (Octopyne type)
Conjugative transfer
Virulence region
Opine catabolism
Origin of replication

SDS PAGE:

When proteins are separated by electrophoresis through a gel matrix, smaller proteins migrate faster due to less resistance from the gel matrix. Other influences on the rate of migration through the gel matrix include the structure and charge of the proteins.

In SDS-PAGE, the use of sodium dodecyl sulfate (SDS, also known as sodium lauryl sulfate) and polyacrylamide gel largely eliminates the influence of the structure and charge, and proteins are separated solely based on polypeptide chain length.

SDS is a detergent with a strong protein-denaturing effect and binds to the protein backbone at a constant molar ratio. In the presence of SDS and a reducing agent that cleaves disulfide bonds critical for proper folding, proteins unfold into linear chains with negative charge proportional to the polypeptide chain length.

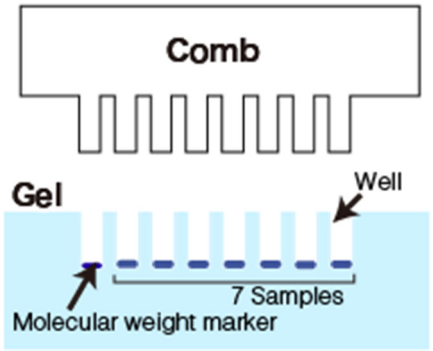
Polymerized acrylamide (polyacrylamide) forms a mesh-like matrix suitable for the separation of proteins of typical size. The strength of the gel allows easy handling. Polyacrylamide gel electrophoresis of SDS-treated proteins allows researchers to separate proteins based on their length in an easy, inexpensive, and relatively accurate manner

PROCEDURE:

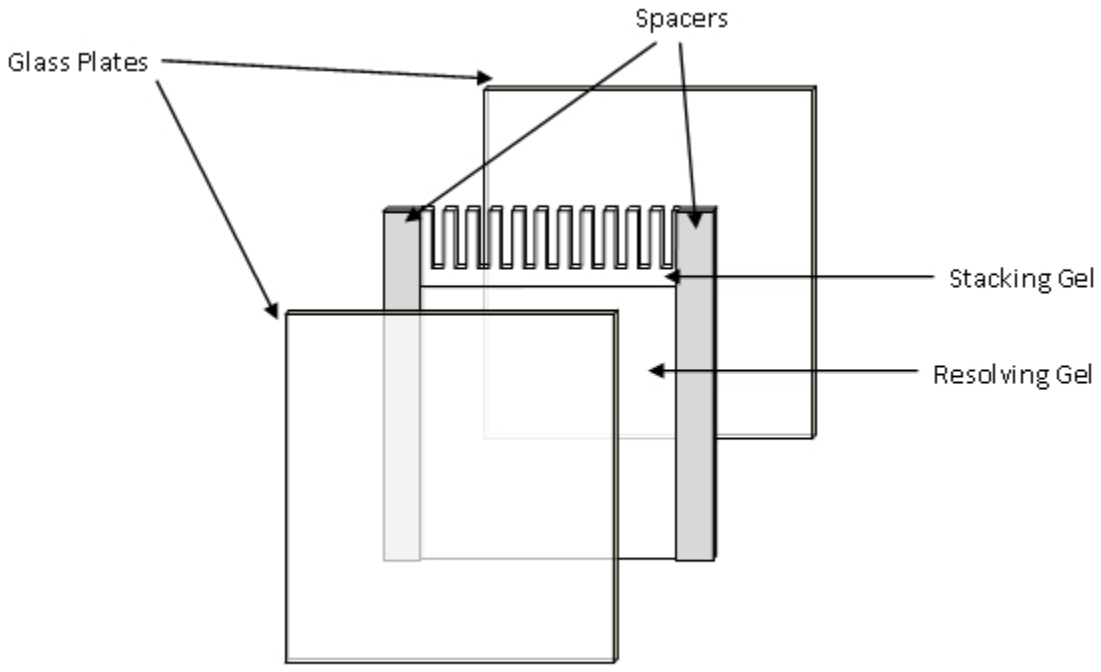
1. Gather combs, glass plates, spacer (silicone tubing), and binder clips.

   A comb is used to make wells (lanes) to load samples. Use an appropriate comb depending on the sample size.

   Example: Use an 8-lane comb for 7 samples and molecular weight markers.



2. Thoroughly clean the glass plates with ethanol, and assemble the gel casting mold.
3. Pour acrylamide solution for a separating gel. Overlay with water to prevent contact with air (oxygen), which inhibits polymerization. Allow acrylamide to polymerize for 20-30 minutes to form a gel. Remove the overlaid water.
   Proteins migrate at different rate depending on the concentration of the separating gel. Use an appropriate gel concentration for your target protein. Using a higher acrylamide concentration produces a gel with a smaller mesh size suitable for the separation of small proteins. In general, an acrylamide concentration between 6 and 15% is used.
   Gels with an acrylamide concentration gradient (gradient gels) are also used.
4. Pour acrylamide solution for a stacking gel; insert a comb and allow the acrylamide to polymerize.
   Proteins are highly concentrated when they migrate through a stacking gel prior to entering a separating gel. The concentration occurs due to the difference in the rate of migration of glycine ion, chloride ion, and proteins.



SDS-Page Gel Setup

5. Add sample buffer to samples, and mix by flicking the tube.

   Heat the samples at 100°C for 3 minutes in a heat block.

Centrifuge at 15,000 rpm for 1 minute at 4°C, and use the supernatant for SDS-PAGE.

6. Remove the binder clips, spacer, and comb from the gel assembly, and mount the gel in the electrophoresis apparatus using binder clips.

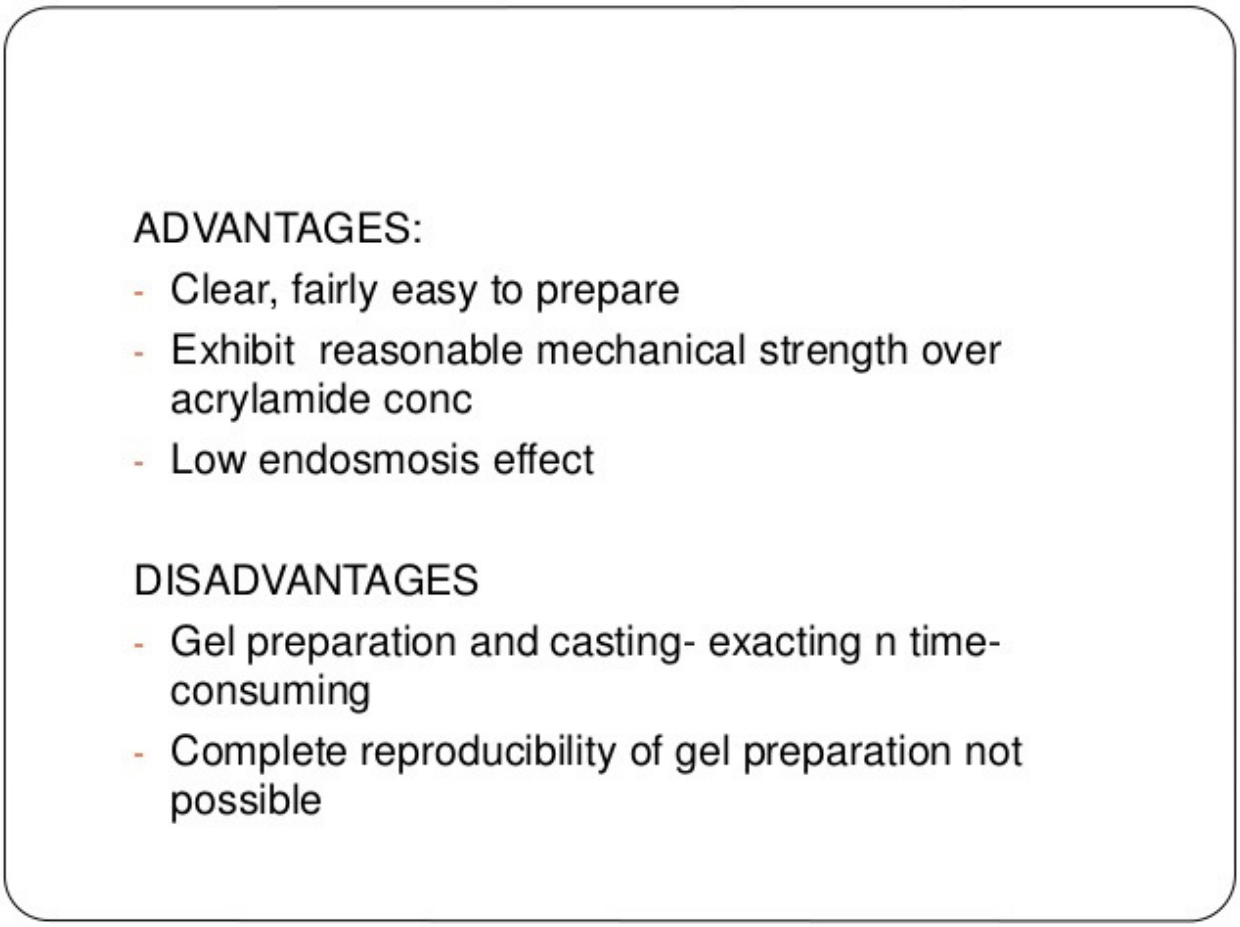
   Pour running buffer into the upper and lower chambers of the electrophoresis apparatus, and remove air bubbles and small pieces of gel from the wells and under the gel using a syringe.
7. Load samples and molecular weight markers in wells.
8. Turn on the power supply, and run the gel until the dye (BPB) in the sample buffer reaches the bottom of the gel.
9. Remove the gel assembly from the electrophoresis apparatus. Remove the gel from the glass plates using a spatula, and prepare for subsequent analysis.

APPLICATION OF SDS -PAGE

SDS-PAGE is used mainly for the following purposes:

1. Measuring molecular weight.
2. Peptide mapping.
3. Estimation of protein size.
4. Determination of protein subunits or aggregation structures.
5. Estimation of protein purity.
6. Protein quantitation.
7. Monitoring protein integrity.
8. Comparison of the polypeptide composition of different samples.
9. Analysis of the number and size of polypeptide subunits.
10. Post-electrophoresis applications, such as Western blotting.
11. Staining of Proteins in Gels with Coomassie G-250 without Organic Solvent and Acetic Acid.
12. Pouring and Running a Protein Gel by reusing Commercial Cassettes.
13. Selective Labelling of Cell-surface Proteins using CyDye DIGE Fluor Minimal Dyes.
14. Detection of Protein Ubiquitination.
15. SDS-PAGE/Immunoblot Detection of Aβ Multimers in Human Cortical Tissue Homogenates using Antigen-Epitope Retrieval.

ADVANTAE AND DISADVANTAGES:



AGAROSE GEL ELECTROPHORESIS:

Agarose gel electrophoresis is one of the most common electrophoresis technique which is relatively simple and straightforward to perform but possesses great resolving power. The agarose gel consists of microscopic pores that act as a molecular sieve which separates molecules based upon the charge, size and shape.

Agarose gel electrophoresis is a powerful separation method frequently used to analyze DNA fragments generated by restriction enzymes, and it is a convenient analytical method for separating DNA fragments of varying sizes ranging from 100 bp to 25 kb. DNA fragments smaller than 100 bp are more effectively separated using polyacrylamide gel electrophoresis whereas pulse-field gel electrophoresis is used to separate DNA fragments larger than 25 kb. Agarose gel electrophoresis can also be used to separate other charged biomolecules such as RNA and proteins.

The separation medium is a gel made from agarose. Agarose is isolated from the seaweed genera Gelidium and Gracilaria and consists of repeated agarobiose (L- and D-galactose) subunits. During gelation, agarose polymers associate non-covalently and form a network of bundles whose pore sizes determine a gel's molecular sieving properties. In general, the higher the concentration of agarose, the smaller the pore size.

To separate DNA using agarose gel electrophoresis, the DNA is loaded into pre-cast wells in the gel and a current applied. The phosphate backbone of the DNA (and RNA) molecule is negatively charged, therefore when placed in an electric field, **DNA fragments will migrate to the positively charged anode.** Because DNA has a uniform mass/charge ratio, DNA molecules are separated by size.

Procedure

Preparation of Agarose gel matrix

The centerpiece of agarose gel electrophoresis is the horizontal gel electrophoresis apparatus. The gel is made by dissolving agarose powder in boiling buffer solution.

The concentration of agarose in a gel depends on the sizes of the DNA fragments to be separated, with most gels ranging between 0.5%-2%. The solution is then cooled to approximately **55°C** and poured into a casting tray which serves as a mold. A well-former template (often called a comb) is placed across the end of the casting tray to form wells when the gel solution solidifies.

After the gel solidifies, the gel is submerged in a buffer-filled electrophoresis chamber which contains a positive electrode (anode) at one end, and a negative electrode (cathode) at the other. The volume of the buffer should not be greater than 1/3 of the electrophoresis chamber. The most common gel running buffers are TAE (40 mM Tris-acetate, 1 mM EDTA) and TBE (45 mM Tris-borate, 1 mM EDTA).

Sample preparation and loading

Samples are prepared for electrophoresis by mixing them with loading dyes. Gel loading dye is typically made at 6X concentration (0.25% bromphenol blue, 0.25% xylene cyanol, 30% glycerol). Loading dyes used in gel electrophoresis serve three major purposes:

1. add density to the sample, allowing it to sink into the gel.

2. provide color and simplify the loading process.
3. the dyes move at standard rates through the gel, allowing for the estimation of the distance that DNA fragments have migrated.

These samples are delivered to the sample wells with a clean micropipette (variable automatic micropipette is the preferred one).

Applying electric current and separating biomolecules

A direct current (D.C.) power source is connected to the electrophoresis apparatus and electrical current is applied. Charged molecules in the sample enter the gel through the walls of the wells. Molecules having **a net negative charge** migrate towards the **positive electrode (anode)** while net **positively charged molecules** migrate towards the **negative electrode (cathode).** The buffer serves as a conductor of electricity and to control the pH, which is important to the charge and stability of biological molecules. Since **DNA has a strong negative** charge at neutral pH, it migrates through the gel towards the **positive electrode** during electrophoresis.

The bluish-purple dye allows for visual tracking of sample migration during the electrophoresis. The gel is run until the dye has migrated to an appropriate distance.

Visualization

The agarose gel will have to be post stained after electrophoresis. The most commonly used stain for visualizing DNA is ethidium bromide (EtBr)*. Alternative stains for DNA in agarose gels include SYBR Gold, SYBR green, crystal violet and methyl blue. The sensitivities of methylene blue and crystal violet are low compared with ethidium bromide. SYBR gold and SYBR green are highly sensitive but more expensive than EtBr.

EtBr works by intercalating itself in the DNA molecule in a concentration-dependent manner. When exposed to short wave ultraviolet light source (transilluminator), electrons in the aromatic ring of the ethidium molecule are activated, which leads to the release of energy (light) as the electrons return to ground state. This allows for an estimation of the amount of DNA in any particular DNA band based on its intensity.

The exact **sizes** of separated DNA fragments can be determined by plotting the log of the molecular weight for the different bands of a DNA standard

against the distance travelled by each band. The DNA standard contains a mixture of DNA fragments of pre-determined sizes that can be compared against the unknown DNA samples.

APPLICATION OF GEL ELECTROPHORESIS:

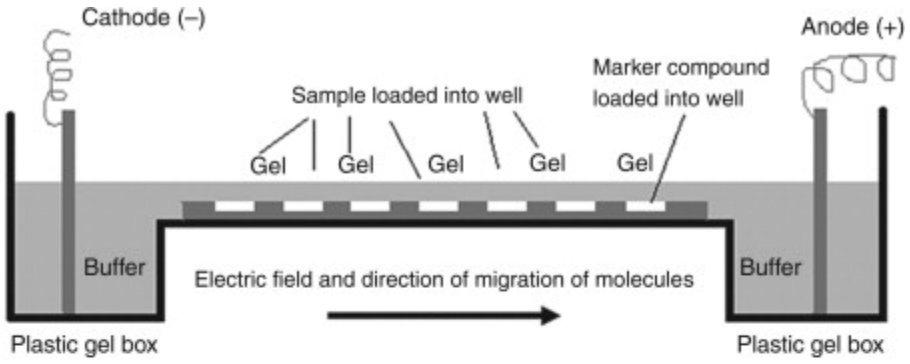
Applications of Agarose Gel Electrophoresis

- Separation of restriction enzyme digested DNA including genomic DNA, prior to Southern Blot transfer. It is also often used for separating RNA prior to Northern transfer.
- Analysis of PCR products after polymerase chain reaction to assess for target DNA amplification.
- Allows for the estimation of the size of DNA molecules using a DNA marker or ladder which contains DNA fragments of various known sizes.
- Allows the rough estimation of DNA quantity and quality.

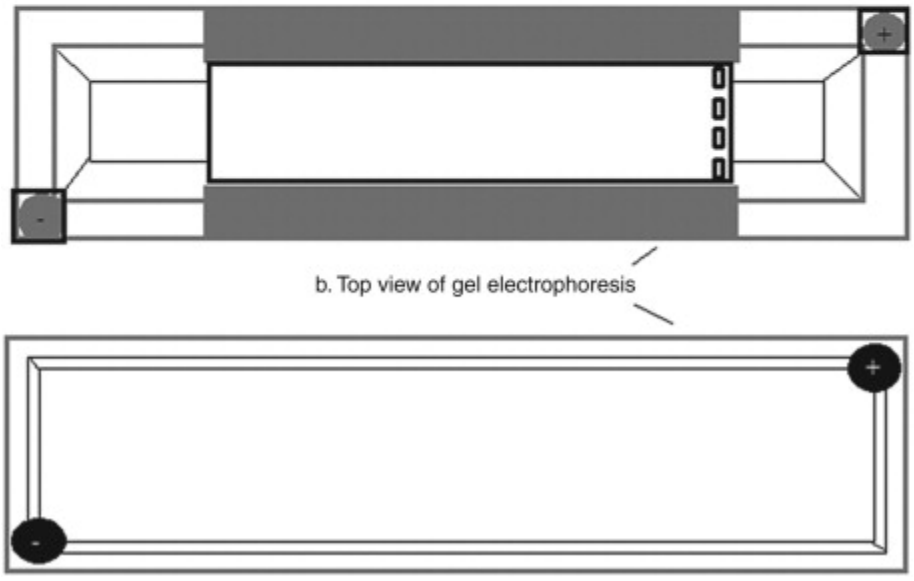
ADVANTAGES AND DISADVANTAGES OF AGAROSE GEL ELECTROPHORESIS:

Advantages	Disadvantages
Nontoxic gel medium	High cost of agarose
Gels are quick and easy to cast	Fuzzy bands
Good for separating large DNA molecules	Poor separation of low molecular weight samples
Can recover samples by melting the gel, digesting with enzyme agarose or treating with chaotropic salts	

Table 1. Advantages and disadvantages of agarose gel electrophoresis.



a. Side view of gel electrophoresis



b. Top view of gel electrophoresis

Gel Type	Agarose	Polyacrylamide
Composition	Agarose is a complex sugar (polysaccharide) derived from seaweed. Agarose gels are made of long chains of interlinked sugars to create a meshwork (like a spiderweb).	Acrylamide is made by digesting acrylonitrile with nitrile hydratase. PA is made by the chemical crosslinking of acrylamide and bis-acrylamide. This linking produces a molecular sieve.
Run Configuration	Horizontally run. The gel will be flat on the table.	Vertically run. The gel is standing on the table.
Casting methodology	Sets as it cools. The agarose comes in a powder that is mixed with buffer and then microwaved. This is poured into the gel frame and allowed to set.	Sets by a chemical reaction once crosslinking occurs.
Properties	The higher the agarose concentration, the smaller the pore sizes. Typically used at concentrations of 0.5-2%.	Porosity is dictated by the ratio of acrylamide to bis-acrylamide. Usually resolving gels are made at a concentration of 6-15%.
Uses	DNA fragments of 50-20,000 bp in size. Resolution of over 6 Mb is possible with pulsed field gel electrophoresis* (PFGE).	PA gels have very high resolving power for small 5-500 bp fragments of DNA. Good for analyzing single-stranded DNA.
Pros	Easily made, poured and handled because the gel setting is a physical rather than chemical change. The gel sets quickly (usually in 30-60 minutes). Samples are easily recovered from the gel. Gels can be refrigerated in a plastic bag or film. The gel is non-toxic and so it is easy to handle (unless ethidium bromide is used as a stain). Pore sizes can be manipulated to increase/decrease molecular sieving. Different buffers can be used to alter resolution and run times. A large range of sizes of DNA can be separated	May give more reproducible results, as it is chemically synthetic and therefore may be more consistent between batches. Pores are uniform in size. Non-reactive with samples since there is no charge associated with it. Thin gels can be cast which can promote better separations at higher field strengths due to efficient heat dissipation. Pore size can be manipulated to increase/decrease molecular sieving. Different buffers can be used to manipulate resolution and run time . Very high resolving power.
Cons	Agarose gels do not have a uniform pore size. Low percentage gels are weak and may break when lifted. High percentage gels are often brittle and may not set evenly.	Acrylamide is a potent neurotoxin. Disposable gloves must be used when handling solutions of acrylamide, and a mask when weighing out powder. Once set, PA is considered to be non-toxic, but PA gels should also be handled with gloves due to the possible presence of free acrylamide. When ethidium bromide is used as a stain, more care should be taken. More care needed when pouring the gel to ensure gas bubbles are removed. Longer wait time for gel to set.